AMENDMENTS TO THE SPECIFICATION

The Office has requested Applicant to cancel the amendment to the specification made in Applicant's communication to the Office dated February 13, 2008. The amendment to the specification set forth herein is intended to comply with this request. Accordingly, please cancel the paragraph in the specification amended in Applicant's communication to the Office dated February 13, 2008, believed to begin at page 19, line 1 of the specification, and replace it with the following paragraph:

> Another aspect which may interplay in the various factors of the present invention is that of utilizing low dose amounts of sperm for artificial insemination or the like. Additional background on the aspect of sexed, artificial insemination may be found in "Prospects for Sorting Mammalian Sperm" by Rupert P. Amman and George E. Seidel, Jr., Colorado Associated University Press (1982) hereby incorporated by reference. As mentioned, natural insemination involves numbers of sperm on the order of billions of sperm. Typical artificial insemination is presently conducted with millions of sperm for bovine species and hundreds of millions of sperm for equine species. By the term "low dose" it is meant that the dosage of sperm utilized in the insemination event are less than one-half or preferably even less than about 10% of the typical number of sperm provided in a typical artificial insemination event. Thus, the term "low dose" is to be viewed in the context of the typical artificial insemination dosage or also as an absolute number. For bovine sperm where currently 1 to 10 million sperm are provided, a low dose process may be considered an absolute number of about 500,000 sperm or perhaps as low as 300,000 sperm or lower. In fact, through utilization of the techniques of the present invention, artificial insemination with good percentages of success has been shown with levels of insemination of sperm at 100,000 and 250,000 sperm (41% and 50%, respectively pregnancy rates). As shown in the article "Uterine Horn Insemination of Heifers With Very Low Numbers of Non-frozen and Sexed Spermatozoa" as published in 48 Theriogenology 1255 (1997) hereby incorporated by reference. ~~In the Abstract, this article includes the following information: "In Experiment 1, semen from 3 Holstein bulls was extended to 1 x 10^5 or 2.5 x 10^5 sperm/0.1 ml; 2.5 x 10^6 total sperm/0.21 ml were used for control. Semen was cooled to 5°C, packaged into modified 0.25-ml French straws, and used 26 to 57 h after collection. Spermatozoa were inseminated 24 h after detection of estrus into the uterine horn of Holstein heifers ipsilateral to the ovary with the largest follicle, as determined by ultrasound 12 h after estrus was detected; side of ovulation was verified by detection of a corpus luteum (CL) by ultrasound 7 to 9 d post estrus. Pregnancy was determined~~

40 to 45 d post estrus. The side of ovulation was determined correctly in 262 of 286 heifers (92%), and pregnancy rates were nearly identical for ipsilateral and contralateral inseminations. Pregnancy rates were 48/118 (41%), 56/111 (50%), and 35/57 (61%) for 1 x 10^5, 2.5 x 10^5 and 2.5 x 10^6 sperm per insemination (P<.05 between 1 x 10^5 and 2.5 x 10^6). There were no significant differences in pregnancy rates (P>.05) among the heifers for the 3 AI technicians or the 3 bulls." On page 1257, this article includes the following information: "Materials and Methods. Experiment 1: Liquid Semen. Semen from 3 Holstein bulls of above average fertility based on nonreturn rates of inseminated females was collected using an artificial vagina and processed at Atlantic Breeders Cooperative, Lancaster, Pennsylvania. Neat semen was treated with antibiotics (24) and extended in Cornell Universal Extender (CUE; 14.5 g/l Na citrate $2H_20$, 2.1 g/l $NaHCO_3$, 0.4 g/l KCl, 3.0 g/l glucose, 9.37 g/l glycine, 0.87 g/l citric acid and 200 ml/l egg yolk) containing 250 µg/ml Gentamycin, 50µg/ml Tylosin tartrate, 150/300 µg/ml Lincospectin and 5% homologous seminal plasma. Extender was prepared the day prior to use, allowing large particles to settle overnight so that the supernatant could be used. Seminal plasma was obtained by centrifuging previously collected ejaculates twice at 3000 x g and stored in liquid nitrogen. Semen was initially extended 1:10 in CUE containing twice the final antibiotic concentration and cooled to 5°C over 2.5 h. After reaching 5°C, semen was further extended 1:1 with CUE containing 5% final antibiotic concentration. The semen was then further extended serially with CUE containing the final antibiotic concentration and seminal plasma to equal insemination doses of 1 x 10^5 and 2.5 x 10^5/0.1 µl spermatozoa or the control insemination dose of 2.5 x 10^6 sperm/0.21ml. Sperm concentrations were verified using hemocytometer. The processed semen was shipped at 5°C to Colorado by overnight commercial courier. Holstein heifers 13 to 15 mo of age and weighing 350 to 450 kg were injected twice at 12-d intervals with 25 mg $PGF_{2\alpha}$ (Lutalyse®, Upjohn, Kalamazoo, MI) to synchronize estrus. Heifers were tail-painted and observed for estrus at 12-h intervals beginning 36 h after the second injection and continuing through 84 h post injection. Ovaries were examined by ultrasonography (Aloka 500, Corometrics, North Wallingford, CT) with a 5-MHz linear probe 12 h after the first detected estrus to determine which ovary had the largest follicle that would most likely ovulate. Inseminations were performed 24 h after the initial detection of estrus with embryo transfer straw guns and stainless steel-tipped side-opening blue sheaths (IMV, Minneapolis, MN). Semen was deposited between the greater curvature and tip of the uterine horn ipsilateral to the presumed preovulatory follicle 26 to 57 h post collection. The experiment was performed in 5 replicates and was balanced over 3 technicians, each inseminating approximately equal numbers of heifers within each bull-sperm number subgroup. To obtain more information with the lower experimental sperm doses, only half as many inseminations

were planned for the controls. The ovary that actually ovulated was confirmed by ultrasonography of luteal tissue 7 to 9 d post insemination. Pregnancy status was also determined by ultrasonography 40 to 45 d post estrus. Data were analyzed by Chi-square with the Fisher-Yates correction for single degree of freedom comparisons." On page 1258, the article contains the following information: "Results. Experiment 1. Pregnancy rates are shown in Table 1. Side of ovulation was predicted correctly in 262 of 286 heifers (92%). However, there was no difference (P>.01) in pregnancy rates between inseminations ipsilateral and contralateral to the side of ovulation. Pregnancy rates differed (P<0.5) between the 2 doses, 1 x 10^5 and 2.5 x 10^6 sperm/inseminate (41 vs 61%), but were not significantly (P>0.1) affected by technician or bull (P>0.1)." On page 1259, the article contains the following table: Table 1. Pregnancy rates (main effects means) with nonfrozen semen (Experiment 1).

Factor	Side [a]	N	% Pregnant
Sperm/inseminate			
1 x 10^5	Ipsilateral	104	41[b]
	Contralateral	14	36
2.5 x 10^5	Ipsilateral	103	50[b, c]
	Contralateral	8	50
2.5 x 10^6 (control)	Ipsilateral	55	62[c]
	Contralateral	2	50
Technician			
A		93	48
B		99	52
C		94	46
Bull			
1		93	51
2		97	51
3		96	45

[a] Side of insemination relative to ovulation.
[b, c] Values without a common superscript differ (P<.05)

On page 1262, the article contains the following information: "The main objective of Experiment 1 was to determine pregnancy rates using sperm numbers just below the threshold for normal fertility but under ideal field conditions. To maximize fertility, we incorporated the following concepts: liquid instead of frozen semen, heifers instead of cows, uterine horn instead of uterine body insemination (17, 21), addition of 5% homologous seminal plasma (1, 5), use of a small inseminate volume (0.1 ml) to minimize effects of dilution (9), prostaglandin synchronization instead of spontaneous estrus (15), insemination ipsilateral to predicted ovulation (13), use of atraumatic, side-opening sheaths instead of standard AI sheaths, inseminating 24 instead of 12 h after detected estrus (14), use of bulls with higher than average fertility, and use of well-trained

~~technicians. Note that some of these concepts, e.g., addition of seminal plasma and late insemination, may be inappropriate under more conventional conditions. We applied these concepts to all inseminations. A limitation of the study is the relatively low numbers of inseminations per sperm dose. However, treatments were balanced over 3 bulls and 3 technicians; pregnancy was determined using ultrasound at 6 wk of gestation rather than from nonreturn data; sperm numbers actually delivered from the straws were confirmed using a hemacytometer; and the work was replicated with 3 ejaculates per bull on separate occasions. Another limitation is that bulls were located 2600 km from the heifers, and timing and temperature conditions of semen shipment were not under our control. Despite these limitations, we clearly demonstrated that pregnancy rates of 40 to 50% are attainable in Holstein heifers using 1 to 2.5 x 10^5 total sperm cells per inseminate. Pregnancy rates with these low sperm numbers were clearly below those of the controls (2.5 x 10^6 sperm/dose), so these very low doses will usually be inappropriate for commercial purposes. However, our work has been followed by logistically easier experiments with low doses of frozen semen (20) that show considerable promise for commercial applications." Returning now to the discussion herein, since~~ Since sperm cells appear to display a sensitivity to dilution, these results may display particular interdependence on the utilization of low dose sperm samples with regards to various techniques of the present invention. The absolute numbers may be species dependent, for equine species, merely less than about ten, five, or even one million sperm may be considered a low dose process.